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Effect of Selected Biocontrol Agents and their Combination with Fungicides on the Mycoflora and Quality of Seeds in Clusterbean.

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ABSTRACT

Among the three biocontrol agents tested, *Trichoderma harzianum* isolate-I and *T. viride* were found to be highly effective in reducing the seed-borne fungi of clusterbean on seed treatement. Their combination with thiram was found to be the next in the order of effectiveness. These biocontrol agents and their combination not only increased the seed germinability significantly but also the seedling vigour.

KEY WORDS : *Trichoderma harzianum, T. viride,* thiram, integrated biocontrol, clusterbean

Clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.), a commonly known vegetable and commercial crop is being increasingly cultivated both in India and abroad. The guar gum extracted from clusterbean (guar) seeds is used in various industries. This crop is attacked by about 40 fungal pathogens, amongst which 15 are seed-borne (Shivanna, 1989). To control these fungal diseases in clusterbean, many fungicides both systemic and non-systemic are being used either as sprays or as seed protectants (Van der Kerk, 1971; Singh *et al.*, 1975; Neergaard, 1977; Karwasra *et al.*, 1979; Sharma, 1983). Some of the fungal pathogens are developing resistance to fungicides (Reddy *et al.*, 1979; Thind and Jhooty, 1987) thereby causing huge damage to crop plants. Since pesticides are causing pollution and toxicity to mammals owing to their non- degradability, newer and other non-conventional methods need to be developed. Biocontrol agents have been effective in controlling several soil-borne fungal diseases (Mukhopadhyay, 1987). In clusterbean, the literature on the use of biocontrol agents or their combination with fungicide is lacking. Hence, in the present investigation, biocontrol agents were applied to seeds individually and in combination with fungicides to study their effect on seed-borne fungi, seed germinability and seedling vigour in clusterbean.

MATERIALS AND METHODS

Seeds of clusterbean cv. Pusa Navbahar harbouring several fungi were taken. From each sample, 100 seeds were randomly drawn four times to represent four replicates.

i) Seed treatment with biocontrol agents : Two isolates of *Trichoderma harzianum* viz., TH-I and TH-II, *T. viride*(TV) and an unidentified species of *Trichoderma* which were isolated from the rhizosphere of clusterbean and found to be antagonistic to four seed-borne pathogenic fungi *viz., Aternaria cyamopsidis, Colletotrichum dematium, Myrothecium roridum* and *Phoma* spp. *in vitro* (Shivanna, 1989) were tested in the present study. The seeds were treated with spores of each antagonist collected from 5-day-old culture grown on potato dextrose agar medium. The spores were made into a paste using 1% gum arabic and coated uniformly over the seeds by shaking them in petri dishes. The spore load was adjusted to approximately 5×10^6 spores/seed.

ii) Seed treatment with combinations of biocontrol agents and fungicides : Two antagonists Viz., TH-I and TV, four fungicides *Viz.*, Carbendazim, Captan, Mancozeb and Thiram were tested. The seeds were first treated with the fungicide at half the recommended dosages (0.12 g/kg for Carbendazim, Mancozeb and Thiram; 0.15 g/kg for Captan) and then with the spores as described above. The untreated seeds served as control. The seeds were tested for the seed-borne fungal incidence by the Standard Blotter method (Anon., 1976). Similarly another set of 100 seeds each were subjected to Ragdoll's method (Anon., 1976) to test for their germinability and seedling vigour. The vigour index (VI) was calculated by using the formula:

VI = average root length + average shoot length x per cent seed germination.

RESULTS AND DISCUSSION

The occurrence of different seed-borne fungi, seed germinability and seedling vigour in treated and untreated seeds are given in Tables 1 and 2. The incidence of *Alternaria alternata* and *A. cyamopsidis* was nil in treatment with TH-I while,

Table 1. Effect of biocontrol agents and their combination with fungicides on seed-borne fungi of clusterbean

Tratments	Per cent incidence of fungi											
	1	2	3	4	5	6	7	8	9	10	11	12
Untreated	25.0A	35.0A	22.0A	10.0A	45.0A	10.0A	12.0A	5.0A	10.0A	20.0A	28.0A	15.0A
TH-I	0	0	1.5EF*	0	2.5F	0	1.5CD	0	1.5BCD	2.5DE	2.0G	5.0D
TH-II	1.5DE	0	3.5D	0	5.5DE	0	2.5C	0	1.5BCD	3.5CD	8.5DE	6.0C
Trichoderma sp.	3.5D	0.5EF	8.0C	2.5C	7.0D	1.5CD	1.5CD	0	8.0A	5.0BC	0.5H	10.0BC
T. viride (TV)	2.0DE	1.5E	4.0D	1.0D	3.5EF	0	1.5CD	0	2.0BCD	2.5DE	2.0G	4.0D
TH-I+Carbendazim	13.0BC	15.0B	12.0B	3.0C	15.0C	2.0C	2.0C	0	1.0CD	5.0C	15.0C	10.0BC
H-I+Captan	11.0C	8.0C	1.0E	0	5.0DE	1.5CD	2.5C	1.0C	1.5BCD	3.5CD	11.0CD	3.0DEF
TH-I+Mancozeb	2.5DE	1.5EF	0.5E	0	3.0EF	0	2.5C	1.0C	2.5BC	4.0CD	5.5EF	4.0D
TH-I+Thiram	1.5E	3.5D	2.5DE	0	0.5G	0	0.5D	0	0	1.0F	2.5G	1.5EF
TV+Carbendazim	15.0B	15.0B	11.5B	5.0B	22.0B	5.0B	5.0B	2.5B	2.0BCD	8.0F	21.03	12.5AB
TV+Captan	12.0BC	6.5C	0	1.0D	7.5D	1.5CD	3.0BC	1.0C	3.0B	5.0BC	8.5DE	4.5DE
TV+Mancozeb	2.0DE	0.5F	0	1.0D	4.0EF	1.5CD	1.5CD	0	1.5BCD	2.5DE	3.5FG	3.5DE
TV+Thiram	2.0DE	0.5EF	0.5EF	0.5D	2.5F	0.5D	0.5D	0	0.5D	1.5EF	2.5G	1.5F

* Values followed by the same letter(s) in a column do not differ significantly at 5% level according to DMRT
TH-I and TH-II - 2 isolates of *Trichoderma harzianum*.
1 - *Alternaria alternata*; 2 - *A. cyamopsidis*; 3 - *Asperqillus flavus*; 4 - *Cladosporium* sp.; 5 - *Colletotrichum dematium*;
6 - *Curvularia lunata*; 7 - *Fusarium equiseti*; 8 - *F. moniliforme*; 9 - *F solani*; 10 - *Myrothecium roridum*;
11 - Non-sporulating fungi; 12 - *Phoma* spp.

TH-II and *Trichoderma* sp. completely eradicated *A. cyamopsidis*. All the combinations of *Trichoderma* except TH-I + Carbendazim and TV + Carbendazim were effective. The combination of biocontrol agents with Thiram and Mancozeb were highly effective.

Different species of *Trichoderma* individually and in combination with fungicides completely eliminated *A. niger* while, *Aspergillus flavus* was totally eradicated on treatment withTV + Captan and TV + Mancozeb. The other combinations and all the biocontrol agents individually reduced the incidence of *A. flavus* significantly. Both the isolates of *T. harzianum* completely eradicated *Cladosporium* spp., while the other biocontrol agents and combination of biocontrol agents with fungicides significantly reduced the incidence. *Curvularia lunata* was totally eliminated on treatment with TH-I, TH-II individually and TH-I + Mancozeb and TH-I + Thiram. Other combinations significantly reduced the incidence.

The seed-borne inoculum of *Colletotrichum dematium* was significantly reduced on treatment with biocontrol agents individually and combination of biocontrol agents with Mancozeb and Thiram. Species of *Colletotrichum* have been earlier reported to be controlled by antagonists like *Bacillus fluorescence* (Novogrudskii *et al.*, 1937), *Acremonium sordidulum* (Singh *et al.*, 1978), *Epicoccum purpurascens* (Singh, 1985) and *Streptomyces olivaceus* (Singh and Sinha, 1986). This is the first report of the efficacy of *T. harzianum* and *T. viride* in eliminating seed-borne pathogenic fungi from clusterbean seeds.

All the biocontrol agents completely eliminated *Fusarium moniliforme* and significantly reduced the incidence of *F. equiseti* and *F. solani*. Among the combinations, TH-I + Thiram completely eliminated *F. moniliforme* and *F. solani*, and reduced the incidence of *F. equiseti* to the maximum extent. The incidence of *Myrothecium roridum* was reduced to the maximum extent on treatment with TH-I and TV and TH-I + Thiram and TV + Thiram among the combinations. The incidence of non-sporulating fungi was also reduced to the maximum extent by the biocontrol agents and their combination with Mancozeb, Captan and Thiram. The incidence of *Phoma* spp., was greatly reduced by TH-I + Thiram and TV + Thiram.

The combination of biocontrol agents with Thiram reduced the incidence of most of the seed-borne fungi of clusterbean. This indicates the insensitivity of both the biocontrol agents to Thiram. The integration of biocontrol agents with fungicides was reported to give significantly higher disease control than that obtained by biocontrol agent or fungicide alone (Mukhopadhyay and Brahmbatt, 1985).

Table 2.. Effect of biocontrol agents and their combination with fungicides on seed germination and seedling vigour of clusterbean.

Treatment	Seed germination (%)	Mean root length (cm)	Mean shoot length (cm)	Vigour index (VI)
Control	87.5CD*	10.00 ± 2.15	9.50 ± 3.86	1706.25
TH - I	90.5AB	10.50 ± 2.12**	9.00 ± 3.03	1840.77
TH - II	88.0CD	10.40 ± 4.10	9.90 ± 3.85	1707.20
T.sp.	92.0A	11.00 ± 2.58	9.90 ± 3.74	1830.80
TV	84.0E	10.90 ± 2.82**	9.60 ± 3.02***	1722.00
TH - I + Cb	88.0CD	8.50 ± 3.44***	7.50 ± 3.54***	1408.00
TH - I + C	89.0BC	9.90 ± 2.11	8.70 ± 3.53***	1655.40
TH - I + M	90.0B	10.00 ± 3.26	9.00 ± 3.76	1710.00
TH - I + T	92.0A	11.50 ± 3.28	10.70 ± 4.20***	2042.40
TV + Cb	85.0E	8.10 ± 2.90***	7.30 ± 2.32	1309.00
TV + C	87.0D	9.76 ± 5.12	8.90 ± 3.06	1632.42
TV + M	85.0E	9.90 ± 3.44	9.00 ± 3.34	1605.50
TV + T	90.0B	11.10 ± 2.82***	10.10 ± 2.62	1908.00

Cb - Carbendazim; C - Captan; M - Mancozeb; T - Thiram

* Values followed by the same letter(s) do not differ significantly at 5% level according to DMRT
Mean root and shoot length values are subjected to 'Z' test after finding standard deviation

** Significant increase over control at 5% level *** Significant decrease over control at 5% level

Trichoderma spp. have been largely used for soil treatment but their bio-efficacy has also led to their use for seed treatment purpose. Seed treatment with TH-I and *Trichoderma* sp. significantly increased the seed germinability while it was significantly decreased by *T. viride*. The biocontrol agents are generally known to increase seed germinability and seedling vigour (Chang-Mew and Kammedahl, 1968). Among the combinations tested, TH-I + Thiram followed by TV + Thiram and TH-I + Mancozeb increased the percentage of seed germination significantly. The increase in seed germinability could be due to the reduction in the per cent incidence of harmful mycoflora of seed.

Root lengths of seeldings obtained from seeds treated with TH-I and *Trichoderma* sp., TH-I + Thiram and TV + Thiram increased significantly while those of seedlings obtained from seeds treated with TH-I + Carbendazim and TV + Carbendazim decreased significantly compared to control. Other combination treatments did not affect root length. The shoot lengths of seedlings did not vary significantly on treatment with biocontrol agents individually or combiantions like TH-I + Mancozeb, TV + Captan, TV + Mancozeb and TV + Thiram when compared to control. The combination treatment of biocontrol agents with Carbendazim resulted in decreased seedling vigour. This could be due to the fungitoxicity of Carbendazim to biocontrol agents. Inhibition of *Trichoderma* by Carbendazim has been reported by Davet (1981). The seed treatment with Carbendazim reuced the germinability in sorghum (Subrahmanya *et al.*, 1988).

TH-I isolate was highly effective in reducing the seed-borne fungal incidence followed by TH-I + Thiram and TV + Thiram. The treatment with these agetns not only increased the seed germinability but also the seedling vigour. Since the fungal pathogens like *A. cyamopsidis, C. dematium, M. roridum* and *Phoma* spp. are known to cause severe damage in clusterbean (Shivanna, 1989), seed treatment with biocontrol agents like *T. harzianum* and *T. viride* alone or in combination with fungicides offer great scope to reduce the incidence of these seed-borne pathogenic fungi.

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REFERENCES

ANONYMOUS, 1976. International Seed Testing Association. International rules for seed testing, Annexes, *Seed Sci. Technol.*, 4, 1-177.

CHANG-MEW, I. and KOMMEDAHL, T. 1968. Biological control of seedling blight of corn by coating kernels with antagonistic micro-organisms. *Phytopathol.*, **58**, 1395-1401.

DAVET, D. 1981. Effects of some pesticides on the colonization of a substance by *Trichoderma harzianum* Rifai in the presence of other soil fungi. *Soil Biol. Biochem.*, **13**, 513-517.

KARWESRA, S.S., GANDHI, S.K. and SAINI, M.L. 1979. Know diseases of your guar crop and save it. *Seeds & Farms*, **5**, 39-40.

MUKHOPADHYAY, A.N. 1987. Biological control of soil-borne plant pathogens by *Trichoderma* spp. *Indian J. Mycol. plant pathol.*, **17**, 1-10.

MUKOPADHYAY, A.N. and BRAHMBATT, A. 1985. Integration of chemical and biocontrol methods for the control of tobacco damping-off (Abst.). *Indian Phytopathol.*, **38**, 610-611.

NEERGARD, P. 1977. Seed pathology. Vol.I & II. The MacMillan press Ltd., London and Basingstoke, 1187 pp.

NOVOGRUDSKII, D., BERESOVA, E., NACHIMOVSKAYA, M. and PERVIAKOVA, M. 1937. The influence of bacterization of flax seed on the susceptibility of seedlings to infection with parasitic fungi. *C.R. Acad. Sci. USSR, N.S.* **14**, 385.

REDDY, M.S., PANDU, S.R., and RAO, A.A. 1979. Effect of using combinations and alternate use of fungicides on the *in vitro* development of fungicide resistance in fungi. *Indian Phytopathol.*, **32**, 591-594.

SHARMA, S.R. 1983. Effect of fungicides on the development of Alternaria blight and yield of clusterbean. *Indian J. Agric. Sci.*, **53**, 932-935.

SHIVANNA, M.B. 1989. Studies on some seed-borne pathogens of clusterbean with special reference to *Colletotrichum dematium* and *Myrothecium roridum*. Ph.D. Thesis, University of Mysore, India, 251 pp.

SINGH, G., GUPTA, R.B.L. and DALELA, G.G. 1975. Efficacy of fungicides and varietal resistance of clusterbean (*Cyamopsis tetragonoloba*) against leaf spot disease caused by *Curvularia lunata*. *Indian Phytopathol.*, **27**, 234-236.

SINGH, R.S. 1985. Use of *Epicoccum purpurascens* as an antagonist against *Macrophomina phaseolina* and *Colletotrichum capsici*. *Indian Phytopathol.*, **38**, 258-262.

SINGH, U. and SINHA, S.K. 1986. A new strain of *Streptomyces olivaceus* and its biological activity (Abst.). *Proc. 73rd Indian Sci. Congr.* part III, Sec. VI, pp. 55-56.

SINGH, U.P., VISHWAKARMA, S.N. and BASUCHAUDHURY, K.C. 1978. *Acremonium sordidulum* mycoparasitic on *Colletotrichum dematium* f. *truncata* in India. *Mycologia*, **70**, 453-455.

SUBRAHMANYA, K.N., PRAKASH, H.S., SHETTY, H.S. and KARANTH, N.G.K. 1988. Effect of Thiram and Bavistin on sorghum seed stored under different conditions. *Pesticides*, **22**, 25-27.

THIND, T.S. and JHOOTY, J.S. 1987. Relative performance of some fungicides in controlling anthracnose and black rot of chillies. *Indian Phytopathol.*, **40**, 543-545.

Van der Kerk, G.J.M. 1971. Systemic fungicides : new solutions and new problems, *Proc. VI Brit. Insectic. Fungic. Conf.* p. 707.